<110> Estell, David Aaron

<120> Human Protease and Use of Such Protease for Pharmaceutical Applications and for Reducing the Allergenicity of Non-Human Proteins

<130> GC532

<140> 09/060,854
<141> 1998-04-15

<160> 6

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<210> 1
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<213> B. amyloliquefaciens

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                                        Val Arg Gly Lys Lys Val
                                          1               5

tgg atc agt ttg ctg ttt gct tta gcg tta atc ttt acg atg gcg ttc      161
Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu Ile Phe Thr Met Ala Phe
            10              15              20

ggc agc aca tcc tct gcc cag gcg gca ggg aaa tca aac ggg gaa aag      209
Gly Ser Thr Ser Ser Ala Gln Ala Ala Gly Lys Ser Asn Gly Glu Lys
        25              30              35

aaa tat att gtc ggg ttt aaa cag aca atg agc acg atg agc gcc gct      257
Lys Tyr Ile Val Gly Phe Lys Gln Thr Met Ser Thr Met Ser Ala Ala
    40              45              50

aag aag aaa gat gtc att tct gaa aaa ggc ggg aaa gtg caa aag caa      305
Lys Lys Lys Asp Val Ile Ser Glu Lys Gly Gly Lys Val Gln Lys Gln
55              60              65              70

ttc aaa tat gta gac gca gct tca gtc aca tta aac gaa aaa gct gta      353
Phe Lys Tyr Val Asp Ala Ala Ser Val Thr Leu Asn Glu Lys Ala Val
                75              80              85

aaa gaa ttg aaa aaa gac ccg agc gtc gct tac gtt gaa gaa gat cac      401
Lys Glu Leu Lys Lys Asp Pro Ser Val Ala Tyr Val Glu Glu Asp His
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                 90                    95                   100
gta gca cat gcg tac gcgcagtccg tgccttacgg cgtatcacaa attaaagccc      456
Val Ala His Ala Tyr
            105


ctgctctgca ctctcaaggc tacactggat caaatgttaa agtagcggtt atcgacagcg      516
gtatcgattc ttctcatcct gatttaaagg tagcaagcgg agccagcatg gttccttctg      576
aaacaaatcc tttccaagac aacaactctc acggaactca cgttgccggc acagttgcgg      636
ctcttaataa ctcaatcggt gtattaggcg ttgcgccaag cgcatcactt tacgctgtaa      696
aagttctcgg tgctgacggt tccggccaat acagctggat cattaacgga atcgagtggg      756
cgatcgcaaa caatatggac gttattaaca tgagcctcgg cggaccttct ggttctgctg      816
ctttaaaagc ggcagttgat aaagccgttg catccggcgt cgtagtcgtt gcggcagccg      876
gtaacgaagg cacttccggc agctcaagca cagtgggcta ccctggtaaa tacccttctg      936
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ctgagcttga tgtcatggca cctggcgtat ctatccaaag cacgcttcct ggaaacaaat     1056
acggggcgta caacggtacg tcaatggcat ctccgcacgt tgccggagcg gctgctttga     1116
ttctttctaa gcacccgaac tggacaaaca ctcaagtccg cagcagttta gaaaacacca     1176
ctacaaaact tggtgattct ttgtactatg aaaaagggct gatcaacgta caagcggcag     1236
ctcagtaaaa cataaaaaac cggccttgga tcaacgtaca agcggcagct cagtaaaaca     1296
taaaaaaccg gccttggccc cgccggtttt ttattatttt tcttcctccg catgttcaat     1356
ccgctccata atcgacggat ggctccctct gaaaattta acgagaaacg gcgggttgac     1416
ccggctcagt cccgtaacgg ccaactcctg aaacgtctca atcgccgctt cccggtttcc     1476
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        <213> B. amyloliquefaciens


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            20                  25                  30
Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala
            35                  40                  45
Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His
        50                  55                  60
Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly
65                  70                  75                  80
Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
                85                  90                  95
Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
            100                 105                 110
Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
        115                 120                 125
Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala
    130                 135                 140
Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly
145                 150                 155                 160
Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala
            165                 170                 175
Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val
            180                 185                 190
Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
```

```
            195                    200                    205
Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser
        210                    215                    220
Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn
225                    230                    235                    240
Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys
                245                    250                    255
Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
                260                    265                    270
Ala Ala Gln
        275


        <210> 3
        <211> 269
        <212> PRT
        <213> B. lentus


        <400> 3
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 1               5                    10                    15
His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                    25                    30
Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                    40                    45
Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
        50                    55                    60
His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                    70                    75                    80
Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                    90                    95
Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                    105                    110
Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
            115                    120                    125
Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
        130                    135                    140
Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                    150                    155                    160
Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                    170                    175
Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                    185                    190
Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
            195                    200                    205
Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
        210                    215                    220
Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                    230                    235                    240
Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                    250                    255
Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                    265


        <210> 4
        <211> 274
        <212> PRT
        <213> B. licheniformis
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Ala Gln Thr Val Pro Tyr Gly Ile Pro Leu Ile Lys Ala Asp Lys Val
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Gln Ala Gln Gly Phe Lys Gly Ala Asn Val Lys Val Ala Val Leu Asp
            20                  25                  30
Thr Gly Ile Gln Ala Ser His Pro Asp Leu Asn Val Val Gly Gly Ala
        35                  40                  45
Ser Phe Val Ala Gly Glu Ala Tyr Asn Thr Asp Gly Asn Gly His Gly
    50                  55                  60
Thr His Val Ala Gly Thr Val Ala Ala Leu Asp Asn Thr Thr Gly Val
65                  70                  75                  80
Leu Gly Val Ala Pro Ser Val Ser Leu Tyr Ala Val Lys Val Leu Asn
                85                  90                  95
Ser Ser Gly Ser Gly Ser Tyr Ser Gly Ile Val Ser Gly Ile Glu Trp
            100                 105                 110
Ala Thr Thr Asn Gly Met Asp Val Ile Asn Met Ser Leu Gly Gly Ala
        115                 120                 125
Ser Gly Ser Thr Ala Met Lys Gln Ala Val Asp Asn Ala Tyr Ala Arg
    130                 135                 140
Gly Val Val Val Val Ala Ala Ala Gly Asn Ser Gly Asn Ser Gly Ser
145                 150                 155                 160
Thr Asn Thr Ile Gly Tyr Pro Ala Lys Tyr Asp Ser Val Ile Ala Val
                165                 170                 175
Gly Ala Val Asp Ser Asn Ser Asn Arg Ala Ser Phe Ser Ser Val Gly
            180                 185                 190
Ala Glu Leu Glu Val Met Ala Pro Gly Ala Gly Val Tyr Ser Thr Tyr
    195                 200                 205
Pro Thr Asn Thr Tyr Ala Thr Leu Asn Gly Thr Ser Met Ala Ser Pro
    210                 215                 220
His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Leu
225                 230                 235                 240
Ser Ala Ser Gln Val Arg Asn Arg Leu Ser Ser Thr Ala Thr Tyr Leu
                245                 250                 255
Gly Ser Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Glu Ala Ala
                260                 265                 270
Ala Gln
```

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    <210> 5
    <211> 275
    <212> PRT
    <213> B. subtilis
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1                 5                   10                  15
His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
            20                  25                  30
Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala
        35                  40                  45
Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His
    50                  55                  60
Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly
65                  70                  75                  80
Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
                85                  90                  95
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Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
        100             105                 110
Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
        115             120                 125
Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser
130                     135                 140
Ser Gly Ile Val Val Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly
145                 150             155                 160
Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala
                165             170                 175
Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala
            180             185                 190
Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
        195             200                 205
Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr
    210             215                 220
Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr
225             230                 235                 240
Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr
            245                 250                 255
Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
            260             265                 270
Ala Ala Gln
    275


    <210> 6
    <211> 1052
    <212> PRT
    <213> Human subtilisin


    <400> 6
Met Lys Leu Val Asn Ile Trp Leu Leu Leu Leu Val Val Leu Leu Cys
1               5               10                  15
Gly Lys Lys His Leu Gly Asp Arg Leu Glu Lys Lys Ser Phe Glu Lys
            20              25                  30
Ala Pro Cys Pro Gly Cys Ser His Leu Thr Leu Lys Val Glu Phe Ser
        35              40                  45
Ser Thr Val Val Glu Tyr Glu Tyr Ile Val Ala Phe Asn Gly Tyr Phe
    50                  55                  60
Thr Ala Lys Ala Arg Asn Ser Phe Ile Ser Ser Ala Leu Lys Ser Ser
65                  70              75                  80
Glu Val Asp Asn Trp Arg Ile Ile Pro Arg Asn Asn Pro Ser Ser Asp
                85              90                  95
Tyr Pro Ser Asp Phe Glu Val Ile Gln Ile Lys Glu Lys Gln Lys Ala
        100             105                 110
Gly Leu Leu Thr Leu Glu Asp His Pro Asn Ile Lys Arg Val Thr Pro
        115             120                 125
Gln Arg Lys Val Phe Arg Ser Leu Lys Tyr Ala Glu Ser Asp Pro Thr
    130             135                 140
Val Pro Cys Asn Glu Thr Arg Trp Ser Gln Lys Trp Gln Ser Ser Arg
145             150                 155                 160
Pro Leu Arg Arg Ala Ser Leu Ser Leu Gly Ser Gly Phe Trp His Ala
            165             170                 175
Thr Gly Arg His Ser Ser Arg Arg Leu Leu Arg Ala Ile Pro Arg Gln
            180             185                 190
Val Ala Gln Thr Leu Gln Ala Asp Val Leu Trp Gln Met Gly Tyr Thr
    195             200                 205
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5

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Gly Ala Asn Val Arg Val Ala Val Phe Asp Thr Gly Leu Ser Glu Lys
    210                 215             220
His Pro His Phe Lys Asn Val Lys Glu Arg Thr Asn Trp Thr Asn Glu
225                 230             235                 240
Arg Thr Leu Asp Asp Gly Leu Gly His Gly Thr Phe Val Ala Gly Val
            245             250             255
Ile Ala Ser Met Arg Glu Cys Gln Gly Phe Ala Pro Asp Ala Glu Leu
            260             265             270
His Ile Phe Arg Val Phe Thr Asn Asn Gln Val Ser Tyr Thr Ser Trp
            275             280             285
Phe Leu Asp Ala Phe Asn Tyr Ala Ile Leu Lys Lys Ile Asp Val Leu
    290             295             300
Asn Leu Ser Ile Gly Gly Pro Asp Phe Met Asp His Pro Phe Val Asp
305             310             315                 320
Lys Val Trp Glu Leu Thr Ala Asn Asn Val Ile Met Val Ser Ala Ile
            325             330             335
Gly Asn Asp Gly Pro Leu Tyr Gly Thr Leu Asn Asn Pro Ala Asp Gln
            340             345             350
Met Asp Val Ile Gly Val Gly Gly Ile Asp Phe Glu Asp Asn Ile Ala
            355             360             365
Arg Phe Ser Ser Arg Gly Met Thr Thr Trp Glu Leu Pro Gly Gly Tyr
    370             375             380
Gly Arg Met Lys Pro Asp Ile Val Thr Tyr Gly Ala Gly Val Arg Gly
385             390             395                 400
Ser Gly Val Lys Gly Gly Cys Arg Ala Leu Ser Gly Thr Ser Val Ala
            405             410             415
Ser Pro Val Val Ala Gly Ala Val Thr Leu Leu Val Ser Thr Val Gln
            420             425             430
Lys Arg Glu Leu Val Asn Pro Ala Ser Met Lys Gln Ala Leu Ile Ala
            435             440             445
Ser Ala Arg Arg Leu Pro Gly Val Asn Met Phe Glu Gln Gly His Gly
    450             455             460
Lys Leu Asp Leu Leu Arg Ala Tyr Gln Ile Leu Asn Ser Tyr Lys Pro
465             470             475                 480
Gln Ala Ser Leu Ser Pro Ser Tyr Ile Asp Leu Thr Glu Cys Pro Tyr
            485             490             495
Met Trp Pro Tyr Cys Ser Gln Pro Ile Tyr Tyr Gly Gly Met Pro Thr
            500             505             510
Val Val Asn Val Thr Ile Leu Asn Gly Met Gly Val Thr Gly Arg Ile
            515             520             525
Val Asp Lys Pro Asp Trp Gln Pro Tyr Leu Pro Gln Asn Gly Asp Asn
    530             535             540
Ile Glu Val Ala Phe Ser Tyr Ser Ser Val Leu Trp Pro Trp Ser Gly
545             550             555                 560
Tyr Leu Ala Ile Ser Ile Ser Val Thr Lys Lys Ala Ala Ser Trp Glu
            565             570             575
Gly Ile Ala Gln Gly His Val Met Ile Thr Val Ala Ser Pro Ala Glu
            580             585             590
Thr Glu Ser Lys Asn Gly Ala Glu Gln Thr Ser Thr Val Lys Leu Pro
    595             600             605
Ile Lys Val Lys Ile Ile Pro Thr Pro Pro Arg Ser Lys Arg Val Leu
    610             615             620
Trp Asp Gln Tyr His Asn Leu Arg Tyr Pro Pro Gly Tyr Phe Pro Arg
625             630             635                 640
Asp Asn Leu Arg Met Lys Asn Asp Pro Leu Asp Trp Asn Gly Asp His
            645             650             655
Ile His Thr Asn Phe Arg Asp Met Tyr Gln His Leu Arg Ser Met Gly
```

6

```
                 660                      665                      670
Tyr Phe Val Glu Val Leu Gly Ala Pro Phe Thr Cys Phe Asp Ala Ser
        675                      680                      685
Gln Tyr Gly Thr Leu Leu Met Val Asp Ser Glu Glu Glu Tyr Phe Pro
        690                      695                      700
Glu Glu Ile Ala Lys Leu Arg Arg Asp Val Asp Asn Gly Leu Ser Leu
705                      710                      715                      720
Val Ile Phe Ser Asp Trp Tyr Asn Thr Ser Val Met Arg Lys Val Lys
                725                      730                      735
Phe Tyr Asp Glu Asn Thr Arg Gln Trp Trp Met Pro Asp Thr Gly Gly
                740                      745                      750
Ala Asn Ile Pro Ala Leu Asn Glu Leu Leu Ser Val Trp Asn Met Gly
        755                      760                      765
Phe Ser Asp Gly Leu Tyr Glu Gly Glu Phe Thr Leu Ala Asn His Asp
        770                      775                      780
Met Tyr Tyr Ala Ser Gly Cys Ser Ile Ala Lys Phe Pro Glu Asp Gly
785                      790                      795                      800
Val Val Ile Thr Gln Thr Phe Lys Asp Gln Gly Leu Glu Val Leu Lys
                805                      810                      815
Gln Glu Thr Ala Val Val Glu Asn Val Pro Ile Leu Gly Leu Tyr Gln
                820                      825                      830
Ile Pro Ala Glu Gly Gly Gly Arg Ile Val Leu Tyr Gly Asp Ser Asn
        835                      840                      845
Cys Leu Asp Asp Ser His Arg Gln Lys Asp Cys Phe Trp Leu Leu Asp
        850                      855                      860
Ala Leu Leu Gln Tyr Thr Ser Tyr Gly Val Thr Pro Pro Ser Leu Ser
865                      870                      875                      880
His Ser Gly Asn Arg Gln Arg Pro Pro Ser Gly Ala Gly Ser Val Thr
                885                      890                      895
Pro Glu Arg Met Glu Gly Asn His Leu His Arg Tyr Ser Lys Val Leu
                900                      905                      910
Glu Ala His Leu Gly Asp Pro Lys Pro Arg Pro Leu Pro Ala Cys Pro
        915                      920                      925
Arg Leu Ser Trp Ala Lys Pro Gln Pro Leu Asn Glu Thr Ala Pro Ser
        930                      935                      940
Asn Leu Trp Lys His Gln Lys Leu Leu Ser Ile Asp Leu Asp Lys Val
945                      950                      955                      960
Val Leu Pro Asn Phe Arg Ser Asn Arg Pro Gln Val Arg Pro Leu Ser
                965                      970                      975
Pro Gly Glu Ser Gly Ala Trp Asp Ile Pro Gly Gly Ile Met Pro Gly
                980                      985                      990
Arg Tyr Asn Gln Glu Val Gly Gln Thr Ile Pro Val Phe Ala Phe Leu
        995                      1000                     1005
Gly Ala Met Val Val Leu Ala Phe Phe Val Val Gln Ile Asn Lys Ala
        1010                     1015                     1020
Lys Ser Arg Pro Lys Arg Arg Lys Pro Arg Val Lys Arg Pro Gln Leu
1025                     1030                     1035                     104
Met Gln Gln Val His Pro Pro Lys Thr Pro Ser Val
                1045                     1050
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